Note

Isolation and hydrolysis of alkali-stable inulin

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In a previous publication[1], it was reported that commercial inulin and inulin prepared from dahlia tubers by a standard method[2,3] are degraded by saturated lime-water solutions to the extent of 6 to 24%, depending on the source of the inulin. These preparations could be stabilized to alkaline degradation by reduction with sodium borohydride, which indicated the presence of free reducing ends. Such end groups were attributed to either (*a*) partial cleavage of the molecule by heat or enzyme(s) during isolation, or (*b*) the presence of partially metabolized molecules in the source.

This note confirms that alkaline degradation is due to depolymerization of inulin during isolation by the usual procedure, and presents a method of obtaining alkali-stable inulin that can be assumed to be a preparation of intact molecules terminated at the reducing end with a sucrose-like linkage[2].

Four varieties of dahlias were grown at Murphysboro, Illinois, in 1964. These tubers were harvested within 24 h after a killing frost, washed, and cut into 1–1.5-cm slices. The sliced tubers were divided into portions, one of which was treated by a standard method[2,3] and two of which were treated by the standard method with the substitution of extractions with 2% $Pb(OAc)_2 \cdot 3H_2O$ and 2% mercuric chloride, respectively, for the initial extraction with water. No differences in alkali-stability or yield were noted, although there were four-fold differences in yield among varieties. This result indicated that enzyme-catalyzed hydrolysis is not the principal cause of alkali-lability. Hence, an alternative method of isolation of inulin was worked out in which a minimum of heating of solutions was employed.

Dahlias (3 varieties) were grown at Murphysboro, Illinois, in 1965. These tubers were harvested within 24 h after a killing frost, washed, cut into 1–1.5 cm slices, and frozen. The slices were frozen and thawed three times, and then the juice was poured off; yield: 985 g of juice and 1,655 g of pulp. The pulp was extracted with 2.5 liters of water for 1.5 h at 60°, removed by filtration, and re-extracted with 2.5 liters of water. The filtrates were combined, and concentrated under diminished pressure at 45–50° to ~1,300 ml, and the concentrate was added to the juice; total volume 2,155 ml. This mixture was frozen, then thawed at 5°, and centrifuged. The residue was saved; the supernatant liquor was concentrated under diminished pressure to 550 ml, and the process was repeated. The two residues were combined,

dissolved in the minimum of water, and recrystallized by freezing and thawing 7 times at 5° as before. The inulin was then washed successively with acetone and ether, and dried; yield 32.1 g; moisture content 13.4%.

Alkaline degradation was effected as previously reported[1]. The inulin preparation was 1.4 ±0.2% degraded.

Hydrolysis of the inulin preparation was effected by either oxalic acid or hydrochloric acid at a variety of temperatures, by yeast invertase, and by a dahlia-tuber extract. Analysis of the D-glucose content of the hydrolyzate was made by g.l.c. analysis and by use of D-glucose oxidase. The following procedure was found to give the highest and most reproducible values. One ml of a solution of the inulin preparation (~3.6 mg/ml) was placed in a screw-capped tube, and to the solution was added 1.0 ml of 0.01M hydrochloric acid. The tubes were capped, heated for 3–5 days at 52°, and cooled to room temperature, and Glucostat reagent (Worthington Biochemical Corp., Freehold, New Jersey) was added. Values for degree of polymerization (d.p.) were calculated from the amounts of D-glucose released on hydrolysis, with the assumption that there is one D-glucosyl group (as a sucrose residue) per molecule[2].

From 11 such analyses, the average d.p. was found to be 24.9 (standard deviation 0.735). The d.p. values ranged from 23.8 to 26.2. A molecular weight of 4050 ±162 is indicated. Molecular weights of 3,200 to 8,050 have been reported for inulin prepared by the usual method[2,3] from various sources[2,4–14]; these determinations were made by both chemical and physical methods with, in general, the higher molecular weights being calculated from D-glucose content. From my experience, methods of inulin hydrolysis other than that used in this work give consistently low values for the D-glucose content and, hence, high values for the molecular weight.

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